

IDENTIFICATION OF THE LINOLEIC AND LINOLENIC ACIDS OF BEEF TALLOW

By H. B. KNIGHT, E. F. JORDAN, Jr., AND DANIEL SWERN

(From the Eastern Regional Research Laboratory,* Philadelphia)

Hilditch and Longenecker (1), in an investigation of the component acids of beef tallow, failed to obtain the tetrabromostearic acid, m.p. 115°, characteristic of vegetable oil linoleic acid (*cis,cis*-9,12-linoleic acid), upon bromination of the fraction which contained the octadecadienoic acid. In addition, upon oxidation of this fraction with potassium permanganate, they obtained only small yields of tetrahydroxystearic acids, whereas yields of at least 40 per cent may be expected from the linoleic acid of vegetable oil (2). Failure to obtain the usual identifying tests for *cis,cis*-9,12-linoleic acid was in agreement with results obtained by other workers who studied the octadecadienic acids obtained from numerous other fats of animal origin (3). The octadecadienic acid of lard, however, gives fair yields of the tetrabromostearic acid and the tetrahydroxystearic acids characteristic of the linoleic acid of vegetable oil (3). Since the octadecadienoic acid of beef tallow had been shown by oxidative splitting to be a 9,12-linoleic acid, it was concluded by these earlier workers that it must be a geometrical isomer of vegetable oil linoleic acid.

It seemed surprising to us that there should be a difference between the octadecadienoic acids of beef tallow and lard, and it was considered desirable to reinvestigate the nature of the octadecadienoic acid of beef tallow, and, at the same time, to attempt to characterize the trienoic acid which spectrophotometric analysis had shown to be a usual, although minor, constituent of this fat (4).

EXPERIMENTAL

The best grade of edible beef tallow (fatty acid composition shown in Table I), obtained from the internal organs of the animal, was employed. Its fatty acid composition was calculated from spectrophotometric analysis (4) and the iodine number (48.3). The tallow was converted to fatty acids by the rapid, large scale, laboratory saponification procedure previously reported and the saturated acids and the oleic acid were removed by crystallization from acetone at -20° and -60°, respectively (5). The filtrate acids from the -60° crystallization contained more than 90 per

* One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

cent of the polyunsaturated acids originally present in the tallow. From 20,000 gm. of tallow, 2000 gm. of these filtrate acids (iodine number 105.2, neutralization equivalent 290) were obtained. This material was then further fractionated as shown in the accompanying diagram. The apparatus and technique employed in the low temperature crystallizations have been reported previously (6). It should be pointed out that throughout the entire fractionation procedure distillation was employed in only one step (F-2 to D-2), and this was a rapid, straight run, vacuum distillation at 1 mm. through an 18 inch Vigreux column, which has a very low pressure drop. Distillation was conducted in this manner to avoid overheating and, perhaps, isomerizing the polyunsaturated acids.

Fractions F-3, P-4, and F-4 were analyzed spectrophotometrically (4) for polyunsaturated acids; their composition is shown in Table II.

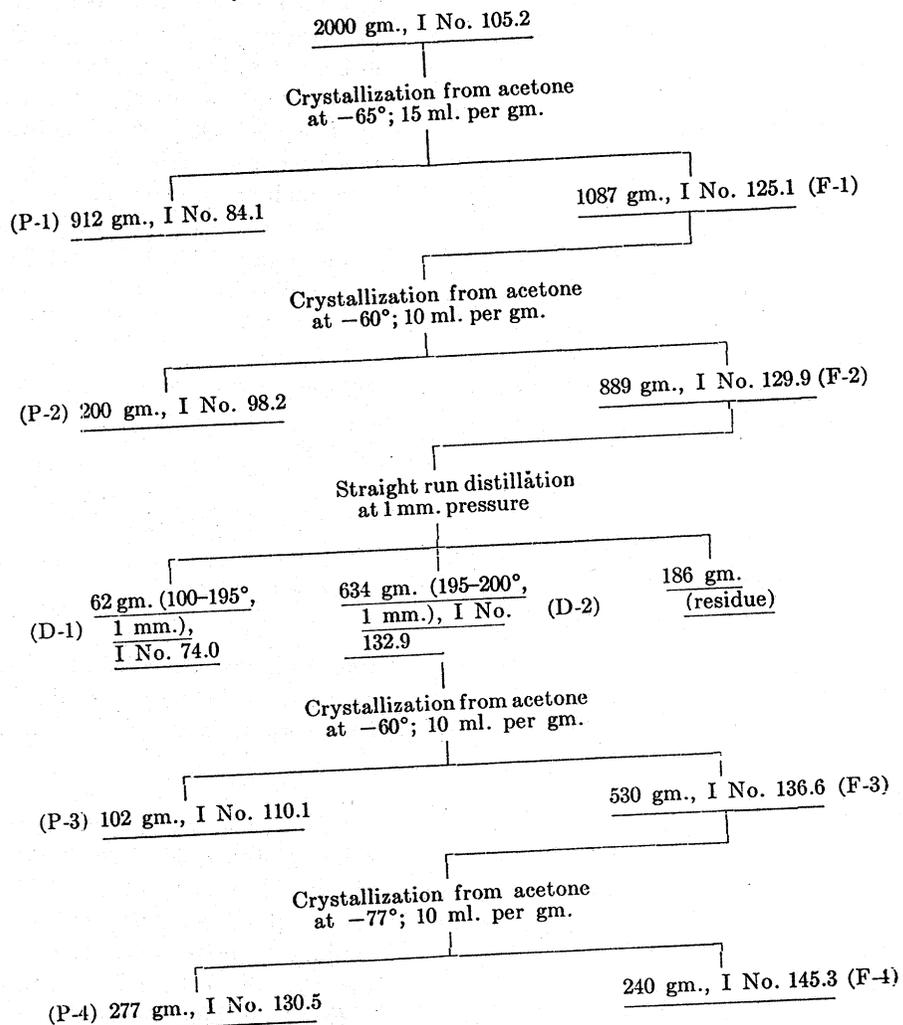
TABLE I
Fatty Acid Composition of Edible Beef Tallow Employed As Starting Material

	<i>per cent</i>
Oleic acid.....	46.3
Saturated acids.....	50.3
Non-conjugated diene acids.....	2.2
" triene ".....	0.46
" tetraene acids.....	0.09
Conjugated diene acids.....	0.66
" triene ".....	0.025
" tetraene acids.....	0.002

Bromination and Isolation of Solid Bromides—To a well stirred solution of 95 gm. of Fraction P-4 (Table II and diagram) dissolved in 2000 ml. of petroleum ether (boiling range, 30–75°) and cooled to –5° to –10°, 86 gm. of bromine were added dropwise over a 20 minute period, during which the temperature rose to about –3°. A white solid precipitated during the addition of bromine. The mixture was stirred for ½ hour at –5° after the bromine addition was complete. Amylene was added until the bromine color had disappeared; the solution was kept at –20° overnight, after which it was filtered by suction. After the precipitate was washed with 500 ml. of petroleum ether at –20°, it was a white, crystalline solid and weighed 33.9 gm. Its melting point was 109–115°, a small quantity remaining unmelted to 133°. This was finely ground and washed several times with boiling ethyl ether to separate it into an ether-soluble (30.8 gm.) and an ether-insoluble portion (3.1 gm.). The ether-insoluble portion, m.p. 171–174°, was crystallized twice from xylene, yielding 1.8 gm. of 9,10,12,13,15,16-hexabromostearic acid, m.p. 180°. When it was

mixed with the hexabromostearic acid prepared from perilla oil fatty acids, the melting point was unchanged.

*Fractionation of Polyunsaturated Acid Fraction Obtained from 20,000 Gm. of Edible Beef Tallow**



* P = precipitate; F = filtrate; D = distillate.

The ether-soluble portion was crystallized at -20° from a mixture of ethyl ether and petroleum ether, yielding 24.1 gm. of 9,10,12,13-tetrabromostearic acid, m.p. $112-112.7^{\circ}$. When it was mixed with the tetra-

bromostearic acid obtained from corn oil fatty acids, the melting point was unchanged.

Fraction F-4 (95 gm.) was brominated as described above with 96 gm. of bromine; 38.1 gm. of insoluble bromides, m.p. 123–139°, were obtained. This precipitate was separated into an ether-soluble portion (25.5 gm.) and an ether-insoluble portion (12.6 gm.). The ether-insoluble portion was crystallized twice from xylene (small quantities of bromides insoluble in boiling xylene were discarded), yielding 5.1 gm. of 9,10,12,13,15,16-hexabromostearic acid, m.p. 170–171.5°, with a small amount remaining unmelted at 198°. When it was mixed with the hexabromostearic acid prepared from perilla oil fatty acids, the melting point was unchanged. The small amount remaining unmelted at 198° probably consisted of bromides of acids containing more than three double bonds.

The ether-soluble material from the bromination of Fraction F-4 was crystallized from a solution of ethyl ether and petroleum ether. The yield

TABLE II
Spectrophotometric Analysis of Fractions F-3, P-4, and F-4 for Polyunsaturated Acids

Fraction	Non-conjugated acids			Conjugated acids		
	Diene	Triene	Tetraene	Diene	Triene	Tetraene
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
F-3	24.7	7.5	0.91	4.6	0.13	0.007
P-4	26.3	5.0	0.23	4.4	0.10	0.004
F-4	23.5	9.5	1.8	5.8	0.20	0.008

was 13.2 gm. of 9,10,12,13-tetrabromostearic acid, m.p. 108.5–113°. When the substance was mixed with the tetrabromostearic acid obtained from corn oil fatty acids, the melting point was unchanged.

DISCUSSION

On the basis of the spectrophotometric analysis (Table II), it is possible to calculate the yield of solid tetrabromostearic and hexabromostearic acids obtainable from Fractions P-4 and F-4, assuming that only 50 and 25 per cent of the theoretical yields of these acids can be obtained from the non-conjugated diene and triene acids, respectively (7, 8). These yields are compared, in Table III, with those actually obtained.

From the high yields of solid bromides shown in Table III, one must conclude that the non-conjugated octadecadienoic and trienoic acids of beef tallow consist mainly of *cis,cis*-9,12-octadecadienoic and *cis,cis,cis*-9,12,15-octadecatrienoic acids, respectively. Stated another way, the major proportion of the 9,12-linoleic and 9,12,15-linolenic acids of beef

tallow has the same geometrical configuration as the linoleic and linolenic acids of vegetable oil. Our data do not preclude the possibility that beef tallow contains other non-conjugated isomers of linoleic and linolenic acids in small proportions, since our bromide yields were always less than expected. Low yields of solid bromides might be expected, however, even if all the non-conjugated linoleic and linolenic acids present were identical with vegetable oil linoleic and linolenic acids, since the yield of solid bromides is affected markedly by the other component acids of the mixture under investigation and low yields are clearly related to the concentration of oleic and saturated acids, which interfere with the complete precipitation of the solid bromides (9).

Also worthy of note is the fact that the iodine numbers of Fractions F-3, P-4, and F-4 as calculated from the spectrophotometric data are 3.8, 2.6, and 6.6 units lower than the iodine numbers determined. In calculating

TABLE III
Calculated Yields of 9,10,12,13-Tetrabromostearic and 9,10,12,13,15,16-Hexabromostearic Acids and Yields Obtained from 95 Gm. Each of Fractions P-4 and F-4

Fraction	9, 10, 12, 13-Tetrabromostearic acid		9, 10, 12, 13, 15, 16-Hexabromostearic acid	
	Calculated	Found	Calculated	Found
	gm.	gm.	gm.	gm.
P-4	26.7	24.1	3.3	1.8
F-4	23.8	13.2	6.3	5.1

the iodine number from the spectrophotometric data, it was assumed that the Wijs solution adds to all the double bonds in the conjugated acids and that no saturated acids were present. The large excess of Wijs solution with respect to conjugated acids makes the first assumption reasonable. The discrepancy is greater still, however, when one considers that saturated material is undoubtedly present in these fractions. The discrepancy is not due to inaccuracy of the methods, since on comparison the iodine number of soy bean and cottonseed oils (per cent of oleic and saturated acids known) calculated from spectrophotometric data usually agrees within 1 unit of the iodine number determined. One possible explanation for the discrepancy is that tallow contains small proportions of polyunsaturated acids, which have been concentrated in Fractions F-3, P-4, and F-4, in which the double bonds are too far apart to be conjugated by means of alkali under the conditions employed in the spectrophotometric analysis. These acids cannot, therefore, be determined spectrophotometrically, but they must react in the normal manner with Wijs iodine solution.

Also of interest is the fact that the ratio of conjugated diene to non-

conjugated diene fatty acids in the starting material and in Fractions F-3, P-4, and F-4 is surprisingly high. This is somewhat unexpected, but it has been confirmed by examining other samples of tallow spectrophotometrically.

Establishment of the fact that the non-conjugated di- and triunsaturated acids of beef tallow consist primarily of *cis,cis*-9,12-linoleic acid and *cis,cis,cis*-9,12,15-linolenic acid, respectively, is of some significance from a nutritional standpoint, since it would indicate that beef tallow (as well as lard) is satisfactory as a source of the so called "essential" fatty acids (the linoleic acid of lard having been shown previously to be *cis,cis*) (3).

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SUMMARY

Crystalline 9,10,12,13-tetrabromostearic and 9,10,12,13,15,16-hexabromostearic acids, identical with those obtained from vegetable oils, have been isolated in good yields from the polyunsaturated acids of edible beef tallow. It has been concluded, therefore, that the non-conjugated octadecadienoic and trienoic acids of beef tallow consist mainly of *cis,cis*-9,12-linoleic and *cis,cis,cis*-9,12,15-linolenic acids, respectively.

On concentrates of polyunsaturated acids obtained from edible beef tallow, discrepancy between the iodine values determined and those calculated from spectrophotometric data suggests the possibility of the presence in this fat of minor proportions of polyunsaturated acids with double bonds too far apart to be conjugated with alkali under the conditions employed in the spectrophotometric analysis.

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